IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Stavrianopoulos et al.
Serial No.:	08/486,070
Filed:	June 7, 1995
For:	**ARRAYS AND SYSTEMS COMPRISING ARRAYS FOR GENETIC ANALYSES AND OTHER APPLICATIONS**

Group Art Unit: 1631

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DECLARATION OF DR. JAMES G. WETMUR

I, James G. Wetmur, hereby declare as follows:

1. I am presently Professor of Microbiology and Human Genetics at the Mount Sinai School of Medicine (MSSM) in New York City, having held that position since 1994. During my sabbatical in 1992, I was Visiting Scientist at Roche Molecular Systems in Alameda, California. I have also held since 1983 the position of Professor of Microbiology at Mount Sinai. Previously from 1974 to 1982, I was Associate Professor of Microbiology at Mount Sinai. Before coming to Mount Sinai in 1974, I was Assistant Professor of Chemistry and Biochemistry at the University of Illinois, Urbana, Illinois, having held that latter position from 1969-1974. While serving in the United States Army from 1967-1969, I was Chief, Biochemistry Branch, U.S. Army Aeromedical

Research Laboratory at Fort Rucker, Alabama. I also reached the rank of Captain. My professional experience is listed on my *curriculum vitae* (CV) attached as Exhibit 1.

2. My education and training are set forth in my CV (Exhibit 1). I received my B.S. in chemistry from Yale University in 1963 and my doctorate in chemistry from California Institute of Technology in 1967. My thesis advisor at Caltech was Dr. Norman Davidson. While a doctoral student at Caltech, I studied the phenomena of nucleic acid hybridization and hybridization kinetics. My studies culminated in my doctoral dissertation titled "Studies of the kinetics of renaturation of DNA" and presented in 1967. This work served as the basis for a publication the next year titled "Kinetics of renaturation of DNA" [Journal of Molecular Biology 31:349-370 (1968)] and co-authored by my advisor, Dr. Norman Davidson, and me. This paper was honored as a Citation Classic in 1983 by *Current Contents, Life Sciences.* A good deal of my research over the past three decades has involved the study of nucleic acid hybridization and renaturation phenonema, with detailed analysis of hybridization and renaturation kinetics in the liquid phase (single phase) and solid or mixed-phase (two phase) and the related phenomenon of branch migration, including nucleic acid formatting, reactants and reaction conditions. These kinetic studies have been extended to such wide-ranging topics as genetic recombination and PCR [e.g., Gonzalez, S. & Wetmur, J.G. Holliday Junction Branch Migration and Resolution: RuvA, RuvB and RuvC from the hyperthermophile Thermotoga maritima. In: DNA Repair Protocols, Vaughan, P., ed., Humana Press, Totowa, NJ, 2000, pp 107-118; Chen, J., Germer, S., Higuchi, R., Berkowitz, G., Godbold, J. & Wetmur, J.G. Kinetic PCR on pooled DNA: A high efficiency alternative in genetic epidemiologic studies. Cancer Epidemiol. Biomarkers Prev. 11:131-136 (2002)]. I have received a great deal of support for my research over the past years. Six recent grants for my research support (active support) are listed on page 2 of my CV (Exhibit 1).

3. I am the author of over eighty scientific publications which are listed on the last several pages of my CV (Exhibit 1). By reason of my professional research activities, I am intimately familiar with the techniques commonly utilized in nucleic acid hybridization applications, having contributed to the development of many of these techniques. Included among my publications are several review articles and chapters written for textbooks. Some of these publications include Wetmur, J.G. & Sninsky, J.J. Nucleic acid hybridization and unconventional bases. In: PCR Strategies, Innis, M.A., Gelfand, D.H. and Sninsky, J.J., Editors, Academic Press, Inc., San Diego CA, pages 69-83 (1995); Wetmur, J.G. Nucleic Acid Hybrids, Formation and Structure of. In: Encyclopedia of Molecular Biology and Molecular Medicine, Myers, R.A., Editor, VCH Publishers, New York, pages 235-243 (1996) and Wetmur, J.G. Nucleic Acid Hybridization. In: DNA Based Computers III, Rubin, H. and Wood, D.H., Editors, American Mathematical Society, 1999, pages 1-23. As a result of my professional research activities, I was invited to chair the opening session and speak at the first Sequencing by Hybridisation Workshop held in Moscow on 19-20 November 1991 which was sponsored by HUGO (the Human Genome Organisation), the US Department of Energy, the Wellcome Trust and the Human Genome Project of the Russian Federation. I addressed the workshop on the first day on the topic of "Principles and Practice of Oligonucleotide Hybridization." As also indicated in my CV (Exhibit 1), I am named as an inventor on several U.S. patents, including U.S. Patents Nos. 4,868,103 ("Analyte Detection by Means of Energy Transfer"); 5,639,607 ("Determining Susceptibility to Lead Poisoning By Detection Of Polymorphisms in the δ-Amino Levulinate Dehydratase Gene"); 5,840,578 ("Methods for Determining Susceptibility to Lead Poisoning"); 5,877,280 ("Thermostable MutS Proteins"); 5,958,681 ("Branch Migration of Nucleotides"); 6,294,325 ("Cloning and Expression of Thermostable MutL Proteins and Uses Thereof"); and 6,358,685 ("Nucleic Acid Displacer Compositions and Cells Comprising Same").

4. As a student or researcher, I have received several honors, including the Eastman Kodak Prize in Chemistry, Caltech (1967) and Career Scientist Award, Health Research Council of New York City (1975). I also received the award of Academy Fellow by the New York Academy of Sciences in 1985. At the New York Academy of Sciences, I have held several positions, including Vice President (Biological Sciences) from 1986-1988; Member of the Board of Governors and Executive Committee of the Board from 1986-1988; and Committee Chairman: Conferences (1985-1986) and Publications, *Annals New York Academy of Sciences* (1987-1988). I have also been asked to perform several special technical services, including being an Invited Expert Analyst, *Chemtracts - Biochemistry and Molecular Biology*. One of the first articles I reviewed was "Light-directed, spatially addressable parallel chemical synthesis," the basic Affymetrix microarray technology, in Chemtracts – Biochemistry and Molecular Biology 2: 207-210 (1991). To date, on behalf of the NIH, I have served at 39 study section meetings, including four years service on the Genome Study Section, and have site visited several types of microarray operations, including Baylor/Genometrix and Affymetrix. I have also taken part in Department of Energy (DOE) site visits of hybridization array technologies at Argonne National Laboratory in 1993 and 1995. These honors and services are listed on the first page of my CV (Exhibit 1). I am a member in several professional organizations, including the American Society for Biochemistry and Molecular Biology (Federation), American Chemical Society (past section officer), New York Science and Technology Forum, American Society of Human Genetics, American Society for Microbiology, Human Genome Organization, and Sigma Xi. These memberships are listed under the "Professional Activities" section of my CV (Exhibit 1), first page. Over the past two decades, I have been engaged as a consultant by several corporations and organizations, the most recent being listed on the second page of my CV (Exhibit 1). I hold a number of committee assignments at Mount Sinai School of Medicine (MSSM), and I teach a number of courses at MSSM, in addition to acting as advisor to doctoral candidates. Thirteen candidates have received their doctorates with me as their advisor. I am currently the chair of the oversight committee

of the Mount Sinai Microarray Shared Instrument Facility. In this capacity, I have responsibility for developing the capability within the Mount Sinai School of Medicine of using microarray technology in medicinal research. This involves both the acquisition of commercial microarray technology and the development of arrays in-house. I recently received an NIH Research Resources grant to purchase a high-density printer for the facility as well as Howard Hughes Medical Institute support for "Statistical and computational methods for large-scale genomic data analysis." The foregoing information is listed on the second page of my CV (Exhibit 1).

5. Over the past two decades, I have been engaged as a consultant by several corporations and organizations, these being listed on the second page of my CV (Exhibit 1). Among those corporations listed in my CV (Exhibit 1) is Enzo Biochem, Inc., whose subsidiary, Enzo Life Sciences, Inc. (formerly Enzo Diagnostics, Inc.) is the assignee of U.S. Patent Application Serial No. 08/486,070. In 2000, I was retained as an expert witness by Affymetrix, Inc. in its litigation against Oxford Gene Technology Limited (*Oxford Gene Technology, Ltd. v. Affymetrix, Inc., D. Del., Civil Action 99-348 (1999*). Oxford Gene Technology is the owner of U.S. Patent No. 5,700,637 for "Apparatus and Method for Analyzing Polynucleotide Sequences and Method of Generating Oligonucleotide Arrays." Professor Edwin Southern is named as the inventor on U.S. Patent No. 5,700,637. During the course of that litigation, I was deposed as an expert witness and I submitted papers to the Delaware Court on behalf of Affymetrix, Inc.

6. As its consultant, I have been asked by Enzo Biochem, Inc. to review the prosecution history of U.S. Patent Application Serial No. 08/486,070, filed on June 7, 1995. Included for my review were significant portions of the file wrapper for this patent, including the patent specification filed on June 7, 1995 and corresponding to U.S. Patent Application Serial No. 06/732,374, filed on May 9, 1985; the specification for U.S. Patent Application Serial No. 06/461,469, filed on January 27, 1983, from which the '070

Application claims priority; the final October 10, 2001 Office Action; Applicants' April 10, 2002 Amendment Under 37 C.F.R. §1.116; Applicants' June 17, 2002 Supplemental Amendment including the Declaration of Dr. Jannis G. Stavrianopoulos; the September 7, 2000 Office Action; Applicants' March 7, 2001 Amendment Under 37 C.F.R. §1.115; and Applicants' May 8, 2001 Communication For Transmitting Declaration of Cheryl H. Agris, Ph.D., Attorney At Law. The latter document also included the Declaration of Cheryl H. Agris, Ph.D., Attorney At Law. I have also reviewed a set of the pending array claims 1576-1761 which were submitted to the U.S. Patent Office in Applicants' June 17, 2002 Supplemental Amendment to their April 10, 2002 Amendment Under 37 C.F.R. §1.116.

7. The pending array claims, 1576-1761, are directed to an array comprising a non-porous substrate having surfaces. Among these claims, claims 1576 and 1670 are independent.

A. As set forth in independent claim 1576, each surface comprises at least one double-stranded nucleic acid fixed or immobilized thereto, and that at least one nucleic acid strand or a sequence therefrom comprises one or more non-radioactive chemical labels which comprise a non-radioactive signaling moiety or moieties which are quantifiable or detectable. Claim 1576 further recites that at least one nucleic acid strand or a sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface, and that the non-porous substrate comprises siliceous matter or polymeric material. Claims 1577-1669 depend from claim 1576 and are directed to various other embodiments, such as the non-porous siliceous substrate (claims 1577-1581); the non-porous polymeric substrate (claims 1582-1588); surface treatment agent and surface treatment (claims 1589-1600); attachment of nucleic acid strand or sequence (claims 1601-1604); nature of nucleic acids (claims 1605-1607 and 1611-1613); source of nucleic acids (claims 1608-1610); labels and signaling moieties (claims 1614-1660); signal quantification or detection

(claims 1661-1664); transparent or translucent properties (claims 1665-1666); collection or set (claim 1667); and system (claims 1668-1669).

B. Claim 1670 is also independent and it is directed to an array comprising a non-porous substrate having surfaces. In its language, claim 1670 recites that each surface comprises at least one nucleic acid strand fixed or immobilized thereto, and that at least one nucleic acid strand or a sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface, and that the non-porous substrate comprises siliceous matter or polymeric material. Claims 1668-1761 depend from claim 1670 and are directed to various other embodiments, including the non-porous siliceous substrate (claims 1671-1675); the non-porous polymeric substrate (claims 1676-1682); surface treatment agent and surface treatment (claims 1683-1694); attachment of nucleic acid strand or sequence (claims 1695-1696); nature of nucleic acids (claims 1697-1701 and 1705); source of nucleic acids (claims 1702-1704); labels and signaling moieties (claims 1706-1752); signal quantification or detection (claims 1753-1756); transparent or translucent properties (claims 1757-1758); collection or set (claim 1759); and system (claims 1760-1761).

8. I have read the October 10, 2001 and the September 7, 2000 Office Actions, including the rejection of various array claims for new matter. The portion of the October 10, 2001 Office Action (pages 3-5) dealing with the new matter rejection is reproduced below:

> In claims 718-872 and 1111-1265 generic arrays are claimed. These claimed arrays start with the broadest versions in claims 718 and 1111 as only requiring a substrate surface with double-stranded nucleic acid fixed or immobilized thereto with at least one strand labeled as described in said claim. The closest array description, as filed, is given in the specification on page 16, lines 9-27. In this description the array also is limited to glass plates having depressions or wells with denatured

analytes deposited therein, wherein single stranded analytes are fixed to the surfaces of the wells. Chemically labeled probes may then be hybridized to these analytes and subjected to detection of any probe-analyte hybrid. It is noted that the analytes are characterized as being "various" which supports the presence of "different" analytes deposited in each well or depression. It is additionally noted that plastic wells are a disclosed option as given in the bridging sentence between pages 20 and 21 of the instant specification. Polystyrene microfilter wells are described on page 22, lines 10-12, as a solid support. The practice of fixing polynucleotide analytes to conventional microtiter plates is described on page 23 at the start of Example 7. In summary, the array embodiments, as filed, are all at least directed to solid supports with wells or depressions therein. It is lastly noted that instant claims 718 and 1111 do not require either wells or depressions as being the form of the array of analyte fixation sites nor its being either glass or plastic, wherein microtiter arrays are deemed to be made of plastic. Such broader array embodiments which are NEW MATTER, for example, include flat surface arrays or non-glass or non-plastic arrays. Applicants have cited a multitude of passages from the instant application as filed but not one broadens the array practice to that which is claimed and indicated above as thus containing NEW MATTER. It is also noted that the Declaration of Dr. CHERYL H. AGRIS has been reviewed and supports the concept of a solid support with well etc. as has been noted before as having support as filed. However, nowhere in this Declaration has there been pointed to the broadening of array practice wherein "ONE" hybridization fluid or mixture simultaneously washes over all, or even a plurality, of the wells or depressions on such array surfaces. That is, whenever an array of wells etc. are described each well or depression etc. forms its own hybridization reaction mixture. Again, there is no array practice disclosed as filed for "ONE" hybridization fluid or mixture contacting, flowing over, or in any way simultaneously permitting overall array hybridization. Contrary to Declarant's allegations wishing to reduce the essential nature of this array practice broadening, the application, taken as a whole or in detail focuses cleanly on separate hybridization reaction mixtures wherever this level of detail is described and thus is essential regarding the scope of the disclosure of this issue.

9. As described in Sections 2-4 above, I have investigated extensively nucleic acid hybridization and renaturation reactions including their kinetics. In over thirty years of investigating nucleic acids, DNA hybridization and renaturation kinetics, I have examined nucleic acids including DNA from a number of different species using a

number of different and diverse formats, including liquid phase (single phase) and solid or mixed-phase (two phase). I am thoroughly familiar with nucleic acid detection formats and nucleic acid probe technology, having spent the better part of my professional and academic career exploring their use as investigative tools for nucleic acid hybridization and kinetic studies.

10. Based upon my own training, background and experience, I would submit that at the time this application was first filed in January 1983, the relevant art to the array subject matter being claimed would have included modifications to nucleic acids, nucleic acid synthesis, surface treatments for immobilization, hybridization, formatting and detection. A person skilled in this art would have possessed or could have been actively pursuing an advanced degree in organic chemistry and/or biochemistry. Such a skilled person could also be at least approaching the level of a junior faculty member with 2-5 years of relevant experience, or would at least be a postdoctoral fellow with several years of experience. I consider myself to possess the requisite level of skill and knowledge of at least a person skilled in the art to which the present array invention pertains.

11. As the consultant for the assignee and also as a person skilled in the art to which the present array invention pertains, I am making this Declaration in support of Applicants' possession of their array subject matter as set forth in the pending claims (1576-1671) in U.S. Patent Application Serial No. 08/486,070. I am being compensated by Enzo for making this Declaration on behalf of its Applicants. As a skilled person, I generally agree with the statements of Drs. Jannis G. Stavrianopoulos and Cheryl H. Agris, Ph.D., in their respective Declarations. As a skilled artisan, it is my opinion and conclusion that Applicants' claimed array practice is directed to surfaces generally, including planar or flat surfaces, and it is not limited to concave surfaces, such as wells or depressions, or to convex surfaces, such as glass beads. It is also my opinion and conclusion that Applicants' claimed array practice involves single and multiple

hybridization fluids or mixtures, and is not limited to multiple separate fluids or mixtures as is typically found in concave surfaces, such as wells or depressions. To state it in another way, Applicants' claimed array practice as disclosed in the first-filed specification for U.S. Patent Application Serial No. 08/486,070, supports the use of "ONE" hybridization fluid or mixture, thus permitting overall array hybridization. As a skilled person in the art, I respectfully submit that the specification for U.S. Patent Application Serial No. 08/486,070 reasonably conveys that the Applicants had possession of their claimed array subject, as set forth in pending claims 1576-1671, at the time their first application was filed in January 1983. The basis for my reasons follows in the sections below.

12. Listed below are several portions in the specification for U.S. Patent Application Serial No. 08/486,070 that disclose, in my opinion, planar or flat surfaces, and not concave surfaces, such as wells or depressions, or convex surfaces, such as glass beads, for use in Applicants' claimed array invention. In at least three of these portions listed below, the term "polystyrene plate" is used to describe a planar or flat surface in the form of a flat bottom plastic Petri dish. I also note that in Section 14C of his Declaration that was submitted on June 17, 2002 in connection with U.S. Patent Application Serial No. 08/486,070, Dr. Jannis G. Stavrianopoulos stated that the term "polystyrene plates" is recognized in the art to mean flat surfaces, such as flat-bottom polystyrene Petri dishes, unless qualified by other language such as "wells" or even "microtiter wells." To support his statement, Dr. Stavrianopoulos attached eleven publications (Exhibits 15-25), which publications were described or quoted in Sections 15A1-A2 and 15B, pages 18-36. I agree with Dr. Stavrianopoulos's statement as supported by those eleven publications, at least three of which (Exhibits 15-17) were published before the filing of the first application leading to the present U.S. Patent Application Serial No. 08/486,070. These three publications are Mage et al., "Mouse Lymphocytes With And Without Surface Immunoglobulin: Preparative Scale Separation in Polystyrene Tissue Culture Dishes Coated With Specifically Purified Anti-

Immunoglobulin," Journal of Immunological Methods 15:47-56 (1977) [Exhibit 15 to Dr. Stavrianopoulos's Declaration]; Wysocki and Sato, "Panning for lymphocytes: A method for cell selection," Proc. Natl. Acad. Sci. (USA) 75:2844-2848 (1978) [Exhibit 16]; and Landreth et al., "Enrichment of human marrow lymphocytes with monoclonal antibodies to murine antigens," Proc. Natl. Acad. Sci. (USA) 79:2370-2374 (April 1982) [Exhibit 17].

A. Example 5, Page 20, Last Full Paragraph

> Because polystyrene from various batches or sources exhibits different binding capacities, the adherence or fixing of DNA to a polystyrene surface is improved by treating the surface with an amino-substituted hydrophobic polymer or material. Previous experiments demonstrated that addition of duodecadiamine (DDA) to polystyrene resulted in an uniform binding coefficient of ***polystyrene plates*** of different batches. Another technique for improving the fixing or uniformity of the plastic surface for fixing DNA involves treatment of the surface with polylysine (PPL). [emphasis added]

B. Example 5, Page 21, First Full Paragraph

> In a further example of the method, denatured adenovirus 2 DNA, the analyte, was bound to ***polystyrene plates*** as described above. After blocking with Denhardt's formula blocking buffer, several biotinylated probes, B-adeno-2-DNA and lambda DNA were hybridized to the immobilized DNA. To one set of immobilized DNA, no probe was added. The extent of hybridization was determined by means of of the antibody-enzyme reaction as described above. It was observed that only the homologous adeno-2 probe hybridized. This technique demonstrated that in vitro hybridization under these conditions is specific and can be monitored quantitatively by the method of the present invention.
> [emphasis added]

C. Example 6, Page 21, Through Page 22, Line 9

> In further tests, radioactively-labeled DNA was prepared by nick translation with [^{3}H]dATP. The labeled, non-biotinylated denatured DNA [2000 ng to 5 ng] was applied to DDA-coated ***polystyrene plates***. The test samples or plates were not allowed to dry. After incubation at 37°C for periods of 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 18 hours, samples were counted. Binding was maximal after two hours of incubation, however, 50 percent of the originally applied DNA bound

regardless of the concentration, thereby indicating that there is an equilibrium between bound and unbound DNA.

[emphasis added]

D. Example 6, Last Paragraph, Through Page 23, First Paragraph

An improved capability for fixing or immobilization of DNA to non-porous siliceous solid supports, such as glass and plastic, is also provided by treatment with a coating of an epoxy resin. For example, treatment of glass or polystyrene surfaces with commercially available epoxy glues, such as a solution of epoxy glue in ethanol [1 percent w/w] serves this purpose. ***These epoxy solutions are applied to the surfaces or wells***, and the solvent, ethanol, evaporated thereupon at a temperature of 37°C, thereby providing a polyamine polymeric coating on the treated surface. These surfaces were found to absorb ^{3}H-labeled DNA from aqueous solution at pH less than 9.5.

13. The following portions in the specification for U.S. Patent Application Serial No. 08/486,070 discloses, in my opinion, concave surfaces, such as wells, depressions, tubes, cuvettes, for use in Applicants' claimed array invention:

A. Page 13, Last Paragraph, Through Page 14, First Paragraph

Yet another aspect of the method of the present invention involves generating the soluble signal from the probe-analyte hybrid in a device capable of transmitting light therethrough for the detection of the signal by spectrophotometric techniques. Examples of devices useful in the spectrophotometric analysis of the signal include conventional apparatus employed in diagnostic laboratories, i.e., ***plastic or glass wells, tubes, cuvettes or arrangements of wells, tubes or cuvettes***. It may also be desirable for both the solid support to which the analyte is fixed and the device to be composed of the same material, or for the device to function as the support in addition to facilitating spectrophotometric detection.

[emphasis added]

B. Example 1, Page 16, First Full Paragraph

Such treated glass could then be employed in the practice of the method of the invention. ***For example, glass plates provided with an array of depressions or wells*** would have samples of the various denatured analytes being fixed to the surfaces of the wells. Thereupon, polynucleotide probes provided with a chemical label may be deposited in

each of the wells for hybridization to any complementary single-stranded analyte therein. After washing to remove any non-hybridized probe, the presence of any hybrid probe-analyte is detectable according to the method of the present invention. One detection technique as described herein involves the addition of an enzyme-linked antibody or other suitable bridging entity of the label for attachment to the probe. Subsequently a suitable substrate is added to elicit the soluble signal, e.g., a color change or chemical reaction, which is then measured colorimetrically or photometrically.

[emphasis added]

C. Example 2, Page 16, Last Two Lines, Through Page 18, First Full Paragraph

In this procedure, glucosylated bacteriophage T_4 DNA, isolated from E. coli CR63 cultures infected with phage T_4 AM82 [44^- 62^-] and purified to be free of chromosomal DNA, or non-glucosylated, highly purified calf thymus is delivered in 100 µl portions to ***treated glass tubes*** in triplicate set. After 15-30 minutes at room temperature, the solution is removed and the ***tubes*** rinsed generously with PBS·Mg^{++} buffer [100 mM Na-K-PO_4, pH 6.5, 150 mM NaCl and 10 mM $MgCl_2$].

One set of ***tubes*** is checked for the presence of DNA by staining with ethidium bromide [100 µl of 1 Mg/ml solution, 30 minutes in the dark, at room temperature]. The staining solution is removed and the ***tubes*** rinsed and checked by UV light. Both glucosylated labelled and unlabelled DNA "probe" bound to the activated glass surface by the observed red fluorescence characteristic of ethidium bromide.

To another set of ***tubes*** is delivered fluorescein-labelled ConA [100 µl of 0.1 mg/ml in PBS·Mg^{++} buffer]. The Concanavalin A [ConA] is obtained and solubilized in 2.0M NaCl at a concentration of 50 mg/ml, and fluorescein-labelled by reacting ConA with fluorescein isothiocyanate at an FITC to protein molar ratio of 3 to 1 in 0.1M sodium borate solution at a pH of 9.2 and at a temperature of 37°C for 60 minutes. Any unreacted FITC is removed by gel filtration on Sephadex G-50. After 60 minutes at room temperature, the solution is removed and the tubes rinsed and checked under UV light. ConA bound only to glucosylated DNA in tubes containing T_4 DNA.

To the third set of ***tubes*** is delivered 100µl of unlabeled ConA in PBS·Mg^{++} buffer. After 60 minutes at room temperature, the tubes are rinsed free of ConA with 0.2M Imidazole buffer pH 6.5.

Acid phosphatase is then added [0.005 units in 100µl at 0.2 percent phosphatase-free BSA] and the tubes are incubated at room temperature

for 30 minutes. After rinsing with 0.15M NaCl to remove any unbound enzyme, 0.1mM paranitrophenylphosphate in 0.2M Imidazole at pH 6.5 is added and incubation contained for 60 minutes at 37°C. The enzyme reaction is terminated by adding 1.0 ml of 0.5 percent sodium bicarbonate and absorbance is determined at A_{300}.

The resulting observed test results indicate that acid phosphatase, one component of the signalling moiety gives a positive visible color reaction, upon reaction with its chromogen, only in tubes containing "probe" T_4 DNA and bridging moiety, ConA, but was washed off from the tubes which contained only ConA or ConA and calf thymus DNA.

[emphasis added]

D. Example 3, Pages 18-19

In an example of the method of the present invention, phage lambda DNA was employed as the analyte, glucosylated DNA as the labelled probe, ConA as the bridging entity and alkaline phosphatase with paranitrophenylphosphate as the signalling moiety. Bacteriophage lambda, obtained by heat induction of E. coli stain W3350 lysogenic for λC_1857 phage, was employed for the preparation of phage lambda DNA. In these tests, the analyte, phage lambda DNA, was immobilized on an activated glass surface according to the following procedure. After rinsing with buffer, ***glass tubes*** were coated with 100µl of coating solution [50 percent formamide, 5X SSC, 100µg salmon sperm DNA 0.2 precent, polyvinylpyrrolidone, 0.1 percent Triton X-100, 0.2 percent BSA and 0.05 percent SDS] at 42°C for 90-120 minutes. The coating solution was removed and the surface was covered with 100µl of coating solution containing phage lambda DNA.

Phage lambda DNA employed as the probe is nick translated with maltose-triose dUTP to introduce glucosyl residues into the DNA. The glucosylated minutes and rapidly cooled in ice bath immediately before use. The ***tubes*** were then incubated with probe at 42°C for 24 hours. The solution was removed and tubes were rinsed with PBS·Mg^{++} buffer. As described above in example 2, ConA is added to the ***tubes*** in PBS·Mg^{++} buffer. After 60 minutes at room temperature the ***tubes*** are rinsed with 0.2M Imidazole buffer.

Also as described in Example 2, the signalling moiety components, acid phosphatase and paranitrophenyl phosphate, are sequentially introduced into the ***tubes***, to generate the detectable soluble signal. In these tests, the glucosyl moiety of the DNA probe is one bridging moiety of the chemical label, and reacts with and is strongly attracted to the second bridging moiety, ConA. The results indicated that acid phosphatase was not washed off from the ***tubes*** which contained

glucosylated probe, whereas ***tubes*** containing non-labelled probe did not show any enzyme activity. [emphasis added]

E. Example 5, Bridging Paragraph, Pages 20-21

In tests involving the fixing of DNA to a plastic surface, biotinylated DNA (b-DNA) was denatured and aliquoted into Dynatech, Immulon II™ removeable ***wells***. Samples were allowed to dry onto the plastic surface at 37°C. The amount of bound b-DNA was determined by sequential addition of goat anti-biotin antibody and rabbit anti-goat antibody complexed to the signalling moiety, alkaline phosphatase, followed by development with p-nitrophenyl phosphate in diethanolamine buffer, pH 9.6. Enzymatic activity was monitored at 504 nm utilizing the automatic Dynatech Micro ELISA Scanner. This procedure enables quantitation of the amount of bound DNA and therefore the degree of biotinylation. To increase the sensitivity of detection, a fluorogenic substrate such as 4-methylumbelliferyl-phosphate, or its analogues, with companion enzymes, may be used.

[emphasis added]

F. Example 6, Page 22, Second & Third Paragraphs

In other tests, ***polystyrene microfilter wells*** were nitrated using the procedure of Filipsson and Hornby, Biochem. J. 120, 215 (1970). The ***polystyrene wells*** were immersed for 20 minutes in a mixture of concentration nitric and sulfuric acid [41 percent, v/v] cooled to 0°C. The ***wells*** were then washed thoroughly with water and subsequently heated to 70°C in a 6 percent solution of sodium dithionate in 2M potassium hydroxide. After 4 hours, the ***wells*** were washed thoroughly with 0.5M hydrochloric acid and distilled water.

To produce 6-aminohexane linked polystyrene, 6-amino-caproic acid-N-hydroxysuccinimide ester·hydrobromide [5 mg thereof dissolved in 0.2M dimethylformamide prepared by reacting 6-aminocaproic acid·hydrobromide with N-hydroxysuccinimide and recrystallized from isopropylalcohol] was added to 0.1M sodium borate [0.4ml]. Amino-derivitized ***polystyrene microfilter wells*** filled with this solution were allowed to react at room temperature for 4 hours and then washed thoroughly with distilled water. The resulting treated ***wells*** absorbed H-labeled DNA from aqueous solution at pH less than 9.5.

[emphasis added]

G. Example 7, Pages 23-25 (Line 3)

Yet another example of the method of the present invention, including fixing the polynucleotide analyte directly to a ***non-porous solid support***, such as a ***conventional microtiter well***, may be performed according to the procedures outlined below.

Conventional microtiter well plates can be pre-rinsed with 1M ammonium acetate (NH_4OAc), in an amount of 200µls/well. Analyte DNA would be diluted to 10-200ng/50µl in water or 10mM Tris-HCl at pH 7.5 and 1mM EDTA (TE). After boiling for 5 minutes and quick cooling in ice water, an equal volume of 2M NH_4OAc would be added and 50µl of analyte DNA is added per well, giving 5-100ng of analyte DNA per well. After open plate incubation for 2 hours at 37°C, the wells can be sealed and plates stored at 4°C. Alternatively, open plates can be incubated at 37°C until the wells are dry, at which point the plates can be sealed, and stored at 4°C for up to one-two months. Single-stranded analyte DNA is now fixed to the ***wells***.

An alternate method to denature and then fix the analyte DNA to the well is to add 50µl of DNA in TE to wells at a concentration of 10-200ng/50µl. After adding 25µl at 0.9N NaOH and mixing, the plates can be incubated for 10 minutes at room temperature. After adding 25µl of 4M NH_4OAc, the open plate may be incubated at 37°C for 4 hours or until dry and the plates sealed and stored at 4°C until ready to use.

To prepare the plates for hybridization, the ***wells*** would be rinsed twice with 0.3m NaCl, 0.03m sodium citrate (2X SSC) (200 µl/well) buffer regardless of whether the plate was dried or not. Preferably, the wells can be rinsed once with 2X SSC/1% Triton X-100 after the two 2X SSC rinses. Plates should be blotted on absorbent paper before beginning each rinse.

To hybridize the fixed analyte with a probe, the following protocol would be followed. A nick translated probe would be heat denatured and added to a hybridization solution containing 30% formamide (deionized), 2X-4X SSPE (20X SSPE = 3.6 M NaCl, 0.2M $NaPO_4$, pH 7.4, 0.02 EDTA) depending on the GC content of probe, 0.1% SDS, and 5.0% dextran sulfate to give a final concentration of 0.2-1.0 µl probe/ml. An alternative hybridization solution contains 30% formamide (deionized), 2X-4X SSPE, 1.0% Triton X-100, and 5.0% dextran sulfate and 0.2-1.0 µg probe/ml. 100µl of the selected hybridization mixture is added to each well. After sealing the plates, they are incubated at 37°C for a desired time.

The hybridization solution is poured out, or collected by aspiration for reuse if desired. The plates are rinsed twice with 2X SSC and 0.1% SDS or 2X SSC and 0.1% Triton X-100 according to whether the first or second hybridization solution identified above was employed. At this point

two to four stringency rinses of SSC and detergent are preferably performed by heating the buffer to the desired temperature and adding it hot to the ***wells***. Formamide and low SSC or SSPE can be used at 37-40°C to achieve the desired stringency. Following stringency washes, ***wells*** are rinsed twice with 1X SSC or 1X SSC and 0.1% Triton X-100, and the plates are now ready for detection. [emphasis added]

14. The following portion in the specification for U.S. Patent Application Serial No. 08/486,070 discloses, in my opinion, convex surfaces, namely, glass beads, for use in Applicants' claimed array invention:

Example 1, Page 15, Through First Paragraph on Page 16

For purposes of the present invention, on analyte is immobilized on a solid support, preferably a non-porous translucent or transparent support. To effect easy fixing of a denatured single-stranded DNA sequence to a ***glass support***, on exemplary "fixing" procedure may involve pretreating the glass by heating or boiling for a sufficient period of time in the presence of dilute aqueous nitric acid. Approximately forty-five minutes in 5% dilute acid should be adequate to leech boron residues from a borosilicate glass surface. The ***treated glass*** is then washed or rinsed, preferably with distilled water, and dried at a temperature of about 115°C, for about 24 hours. A 10 percent solution of gamma-aminopropyltriethoxysilane, which may be prepared by dissolving the above-identified silane in distilled water followed by addition of 6N hydrochloric acid to a pH of about 3.45, will then be applied to the glass surface. The glass surface is then incubated in contact with the above-identified silane solution for about 2-3 hours at a temperature of about 45°C. The glass surface is then washed with an equal volume of water and dried overnight at a temperature of about 100°C. The resulting treated glass surface will now have available alkylamine thereon suitable for immobilizing or fixing any negatively charged polyelectrolytes applied thereto. [See Weetal, H. H. and Filbert, A. M., "***Porous Glass*** for Affinity Chromatography Applications", Methods in Enzymology, Vol. XXXIV, Affinity Techniques Enzyme Purification: Part B. pp. 59-72, W. B. Jakoby and M. Wilchek, eds.]

[emphasis & footnote added]

Note: A copy of Weetal & Filbert's above-cited 1974 publication is attached to my Declaration as Exhibit 2.

15. My opinion and conclusion that the specification in U.S. Patent Application Serial No. 08/486,070, would have reasonably conveyed Applicants' possession of flat planar surfaces in their claimed array subject matter as well as other non-planar surfaces, such as concave and convex surfaces, is bolstered by the fact that quantification and detection are readily carried out on flat planar surfaces which present a plane of focus for observation and measurement by instrumentation, including photometric instrumentation. These would include detection scanners and readers, including more recently, DNA microarray readers. The specification for U.S. Patent Application Serial No. 08/486,070 contains numerous portions citing to the use of photometric techniques, including spectrophotometric and colorimetric means for detection and quantification. See, for example, page 11, last six lines of the first paragraph; page 11, the first six lines of second paragraph; page 13, middle and last paragraph, continuing through the first paragraph on page 14; and page 16, last four lines of the second paragraph. See also the originally filed claims 2 and 16. See also pending claims 1661-1664 and 1753-1756. The advantages of uniformity or consistency in detection which are obtained using a flat surface for microarrays were recently described by Jorge D. Cortese (2001) ["Microarray Readers: Pushing the Envelope," The Scientist 15:36-40 (December 10, 2001)]:

> The final technical issue is the quality of the microarray itself. If the slide is not perfectly flat, the images will not be uniform or consistent, regardless of resolving power.

A copy of Cortese's article is attached as Exhibit 3.

16. As a final note, I wish to point out that some microarray products have been disclosed that use wells, such as a 96-well microarray format. See, for example, Mendoza et al. (1999) ["High-throughput microarray-based enzyme linked immunosorbent assay (ELISA)," Biotechniques 27:778-780 (October 27, 1999)], copy

attached as Exhibit 4.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

9/4/02
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****

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NIH Study Sections (1990ff):

- Special: Molecular Genetics, SBIR: 1999, 2001; Genome: 1990; 2000; Shared instruments: 1998; Career awards: 1999; NIAID Bioterrorism, 2002; NIHGR: DNA Sequencing: 1992, 1998; Yeast Genome: 1996; NCI: Program Projects: 1994, 1996, 1997.
- Site Visits: Genome: Affymetrix (1992, 1995), Genome Therapeutics (1993), Cold Spring Harbor (1993); NCI: Cornell (1994, 1996), Baylor Medical/Genometrix (1996), Temple/Penn/Molecular Dynamics (1998); NIEHS: Harvard (2002).
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New York Science and Technology Forum
American Society of Human Genetics; American Society for Microbiology;
The Human Genome Organisation; Sigma Xi

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Enzo Biochem, Incorporated, 1984-present
Roche Molecular Systems, Hoffmann La-Roche, hybridization, 1993-present
Academic Medicine Development Company (AMDeC), Microarray Core Advisory Committee, 2001-present
Biosafety Committee, Biomedical Research Alliance of New York (BRANY), 2001-present
Cornell University Medical College, GenVec Oversight Committee, 1997-present

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Member, Conflict of Interests Committee
Chair, Oversight Committee, MSSM Microarray Shared Instrument Facility
Chair, Institutional Biological Safety Committee

Teaching: (Complete courses or course director only; excludes seminar courses)

Microbiology:

Microbiology and infection (medical school microbiology)
Microbial and molecular biology
Research methods for biomedical sciences

Chemistry:

Physical chemistry - for biologists
Physical chemistry - quantum mechanics
Physical chemistry of macromolecules
Physical chemistry laboratory; Physical biochemistry laboratory

Ph.D. Theses Directed: 13

Physical Chemistry - 5; Biochemistry - 1; Microbiology - 6; Human Genetics - 1.

Research Support (active support):

Co-P.I. and Core Leader (Genetic Analysis), Organochlorines and metals in New York and the Hudson, NIH P42 ES07384, 5/1/95-3/31/06.
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P.I., Haplotyping for environmental genomics, NIH R21 ES11643, 3/27/02-2/28/05
P.I., Statistical and computational methods for large-scale genomic data analysis, HHMI, 8/1/02-7/31/04.
Project Director, Project 3, Inner city toxicants and neurodevelopmental impairment, NIH P50 E09584; EPA R827039 , 10/1/98-9/30/03.
P.I., High density printing for the Mt. Sinai microarray facility, NIH S10 RR16723, 4/1/02-3/31/03.

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